

Listing of Claims

The following listing of claims will replace all prior versions, and listings, of claims in the subject application:

1. (currently amended) A method for reproducibly generating dendritic cells, comprising the steps of:

(a) obtaining blood mononuclear cells through collection of monocytes and monocyte precursors, and loading the blood mononuclear cells into a cell culture container containing microcarrier beads and media for culturing dendritic cells therein;

(b) incubating [[, for a predetermined time period, tissue culture comprising]] the contents of the cell culture container, including the media and the blood mononuclear cells loaded in the container in step (a), in order to grow dendritic cell culture; and

(c) ~~separating~~ removing nonadherent cells and cells adhered which do not adhere to the beads after the incubation in step (b), from the cell culture container, by resuspending the contents of the cell culture container, allowing the microcarrier beads in the container to settle, and expressing off the supernatant out of the container.

2. (currently amended) A method for reproducibly generating dendritic cells, comprising the steps of:

(a) loading microcarrier beads and media for culturing dendritic cells into a cell culture container, said media including rh-GM-CSF and at least one of rh-IL-4 and rh-IL-7;

(b) obtaining blood mononuclear cells through collection of monocytes and monocyte precursors, and loading the blood mononuclear cells into the container;

(c) incubating ~~[[~~, for a predetermined time period, tissue culture comprising]] the contents of the cell culture container, including the media and the mononuclear cells loaded in the container in step (b), in order to grow dendritic cell culture; and

(d) ~~separating~~ removing from the cell culture container nonadherent cells ~~and cells adhered~~ which do not adhere to the beads after the incubation in step (b), by resuspending the contents of the cell culture container, allowing the microcarrier beads in the container to settle, and expressing off the supernatant out of the container.

3. (original) The method of claim 1, wherein the container comprises a gas permeable cell culture bag.

4. (original) The method of claim 1, wherein the container is a closed vessel.

5. (currently amended) The method of claim 1, ~~wherein further comprising washing the tissue culture incubated for the predetermined time period in step (b) is washed to remove nonadherent cells.~~

Claims 6-7 (canceled).

8. (currently amended) The method of claim 7 further comprising:

(d) introducing additional media for culturing dendritic cells into the cell culture container after the nonadherent cells are removed from the cell culture container in step (b), said media including rh-GM-CSF and at least one of rh-IL-4 and rh-IL-

(f) ~~incubating the container for a second predetermined time period after the contents of the cell culture container after the additional media are introduced into the cell culture container~~
in step (e) (d);

(g) agitating the contents of the container ~~incubated after~~
the incubation in step (f); and

(h) harvesting cell culture suspension by ~~expression~~
expressing off into transfer bags using a sterile connecting device after the beads agitated in step (g) are allowed to settle.

9. (original) The method of claim 1, wherein after step (c) samples are removed from the container for quality control.

10. (original) The method of claim 9, wherein the quality control includes at least one of viability staining, microbial analysis, cell enumeration, microscopic examination of dendritic cell morphology, and immunophenotyping to determine a purity of the dendritic cell preparation.

11. (original) The method of claim 1, wherein the blood mononuclear cells are obtained by apheresis.

12. (original) The method of claim 1, wherein a ratio of a combined surface area of the microcarrier beads and the container to a volume of the container volume is a value that allows the container to hold enough media for the predetermined time period of incubation in step (b).

13. (new) A method for reproducibly generating dendritic cells, comprising the steps of:

(a) obtaining blood mononuclear cells through collection of

monocytes and monocyte precursors, and loading the blood mononuclear cells along with medium for culturing dendritic cells into a cell culture container containing microcarrier beads therein;

(b) preparing the unprocessed contents of the cell culture container, including the medium and the blood mononuclear cells loaded into the cell culture container in step (a), for culturing, by incubating said unprocessed contents, allowing beads with adherent cells attached thereto after incubation to settle, and then expressing off supernatant including nonadherent cells;

(c) introducing additional media for culturing dendritic cells into the cell culture container after the supernatant is expressed off in step (b), said additional media including rh-GM-CSF and at least one of rh-IL-4 and rh-IL-7;

(d) incubating the contents of the cell culture container after the additional media are introduced into the cell culture container in step (c), in order to grow dendritic cell culture; and

(e) harvesting the dendritic cell culture from the incubated contents of the cell culture container, including agitating said incubated contents, allowing the beads in the cell culture container to settle after said agitating, and expressing off cell culture suspension into another container.